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Mitochondrial Anomalies in a Swiss Family With Autosomal Dominant Myoglobinuria

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We report on a Swiss family in which 10 individuals of both sexes in 4 successive generations suffered from myoglobinuria, precipitated by febrile illness. It is the second family described with autosomal dominant inheritance of myoglobinuria. Four individuals suffered acute renal failure, which in two was reversible only after dialysis. In a recent case, a mitochondrial disorder was suspected because of an abnormal increase in lactate levels during an exercise test and because of a subsarcolemmal accumulation of mitochondria in a muscle biopsy, associated with a lack of cytochrome C oxidase in some muscle fibers. No mutation in the mitochondrial DNA was identified. Along with the inheritance pattern, these findings suggest that the myoglobinuria in this family is caused by a nuclear-encoded mutation affecting the respiratory chain. Am. J. Med. Genet. 69:365–369, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: myoglobinuria; autosomal dominant; mitochondria; cytochrome C oxidase; respiratory chain

INTRODUCTION

Rhabdomyolysis, or the lysis of skeletal muscle cells leading to myoglobinuria, can be due to acquired or hereditary causes. The former include direct muscular injury, alcohol abuse, certain drugs and toxins, heat-related syndromes, and febrile infections, whereas the latter include inherited disorders of glycogen and lipid metabolism, and other genetic diseases such as malignant hyperthermia [Poels and Gabreëls, 1993]. In addition, respiratory chain disorders are now recognized as rare causes of myoglobinuria [Ogasahara et al., 1989; Ohno et al., 1991; Saunier et al., 1995; Keightley et al., 1996; Hirano et al., 1996].

We describe a family in which 10 members in four successive generations suffered from episodes of myoglobinuria, in almost all cases induced by febrile illness. In four individuals these led to acute renal failure. The possible causes of this hereditary myoglobinuria are discussed in the light of the investigation of one affected individual.

CLINICAL REPORT

The family (Fig. 1) is of Swiss origin, with no consanguinity. Individuals of both sexes were affected in at least 4 subsequent generations.

Propositus (V-16)

He was hospitalized in 1994 at age 24 years with acute renal failure due to rhabdomyolysis, occurring two days after onset of a febrile infection with vomiting. On admission, temperature was 38°C and blood pressure 120/80 mm Hg. Muscles were swollen and painful. Auscultation revealed a left basal pneumonia. Laboratory findings were: white blood cell count 24.1 × 10^9/l (normal 4–11); sodium 123 mmol/l (135–148); potassium 5.3 mmol/l (3.1–4.6); calcium 1.27 mmol/l (2.3–2.6); creatinine 472 μmol/l (53–115); creatine kinase (CK) 719,625 U/l (5–270); aspartate aminotransferase (ASAT) 5,691 U/l (14–50). Hemocultures were positive for *S. pneumoniae.* Myoglobinuria and ketonuria were present. He was treated with hemodialysis leading to a rapid improvement of renal function.

Seven months after this acute episode of myoglobinuria, neuromuscular investigations were done. A graded exercise test on a cycle ergometer was performed, with a workload of 200 W attained after 16 minutes and maintained for 15 minutes. The basal lactate level of 0.9 mmol/l (1.0–1.9) increased to 9.2 mmol/l (normal: up to 3× resting value). The basal lactate/pyruvate ratio of 13 (<20) increased to 58. Serum levels of CK, carnitine, and acetyl-carnitine were normal.

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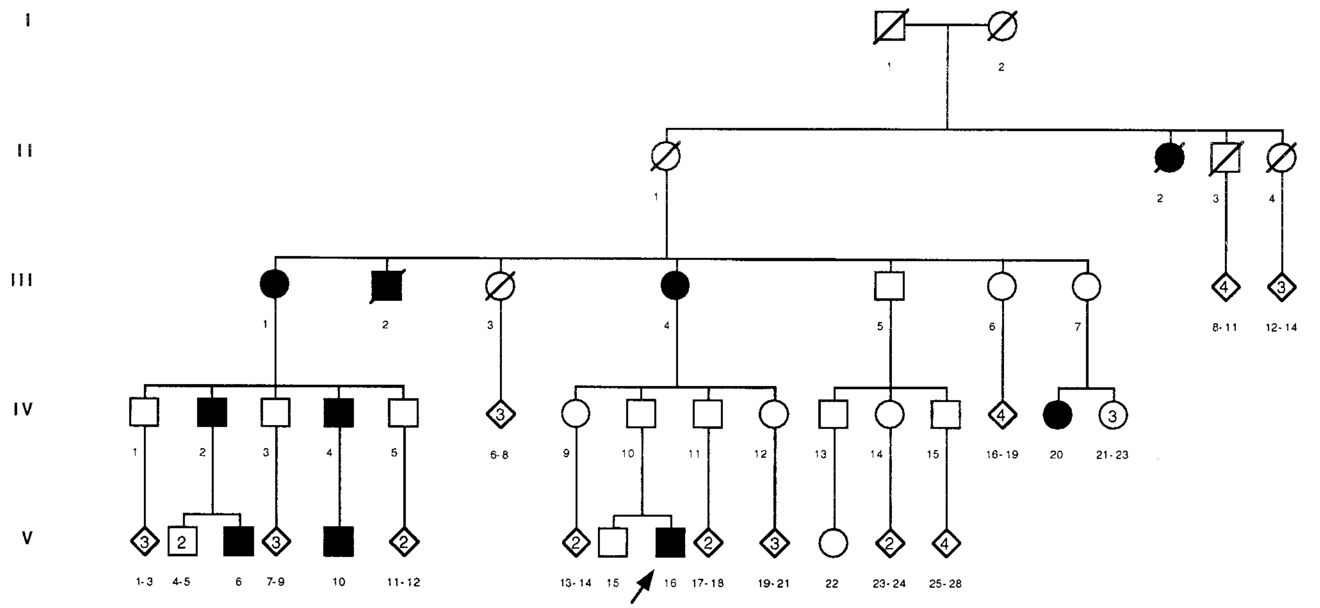

Fig. 1. Pedigree of the family. Filled symbols represent individuals with myoglobinuria. The proband (V-16) is indicated by the arrow.

Basal level of free fatty acids was 690 nmol/l (100–600). Muscle biopsy (quadriceps) showed a normal distribution of glycogen (PAS and PAS diastase staining) and of lipid droplets (Blue Nile staining). NADH and SDH stains revealed a focal subsarcolemmal accumulation and proliferation of mitochondria (Fig. 2A). No ragged red fibers were detected with Gomori trichrome modified staining. Cytochrome C oxidase staining revealed some negative fibers (Fig. 2B).

Carnitine palmitoyltransferase II (CPT II) activity in cultured skin fibroblasts, measured according to Demaugre et al. [1990], was in the normal range.

Leukocyte and muscle mitochondrial DNA (mtDNA) were screened for deletions or duplications and for the commonly-found mtDNA point mutations at positions 3243, 8344, and 8993 [Morris, 1993]. No anomaly was detected. Insufficient biopsy material was available for biochemical investigation of respiratory chain activity.

In addition, no deletions of mtDNA were detected by Southern blot analysis of leukocyte DNA in the other living affected individuals.

Patient II-2

She died in 1906 at the age of 20 years of acute renal failure subsequent to a febrile illness, with muscle pain and emission of dark urine.

Patient III-2

He died in 1932 at the age of 20 years, of acute renal failure after a febrile angina. At autopsy, acute tubular necrosis of the kidneys was found.

Two of his sisters (III-1 and III-4) presented between the ages of 18 and 20 years with urinary tract infection, emission of dark urine, and muscle pain and weakness which lasted approximately 1 month.

Patient IV-2

(Previously Reported in the Thesis of Haissly, [1970]).

He was hospitalized at 30 years with intense muscle pain, brown urine, and high fever due to influenza B infection. Physical examination showed painful muscle swelling, muscular weakness with areflexia, and fever of 39°C. Blood pressure was 120/70 mm Hg. Myoglobinuria was present. Plasma CK was 990 UI/l, ASAT 3400 U/l, potassium 6.2 mmol/l, calcium 1.6 mmol/l, urea nitrogen 166 mmol/l (2.8–8.6). The patient was treated with sodium bicarbonate and mannitol infusion, but nevertheless became anuric and required peritoneal dialysis. Two months later, he left the hospital having lost 18 kg. Creatinine clearance was 16 ml/min (N 60–140). One year later, he had partially recovered his muscle strength and renal function. Upon rehospitalization for a febrile viral infection, neither muscle pain nor myoglobinuria was noted. Electromyography demonstrated small potentials suggesting a diffuse muscular disease. Histochemical analysis of a muscle biopsy showed normal glycogen and acid maltase content. Biochemical investigation documented normal phosphorylase levels; phosphorylase kinase was increased, but showed normal affinity for phosphorylase.

Patient IV-4

He was 17 years old when he presented with muscle pain and myoglobinuria, 7 days after appendicectomy. He had no fever. Plasma creatinine was 113 μmol/l, and ASAT 945 U/l. He received a perfusion of bicarbonate solution and oral vitamin E (200 mg/day). Muscular pain disappeared and the urine became clear. Three years later (in 1964) a similar episode occurred following moderate physical effort several days after an in-

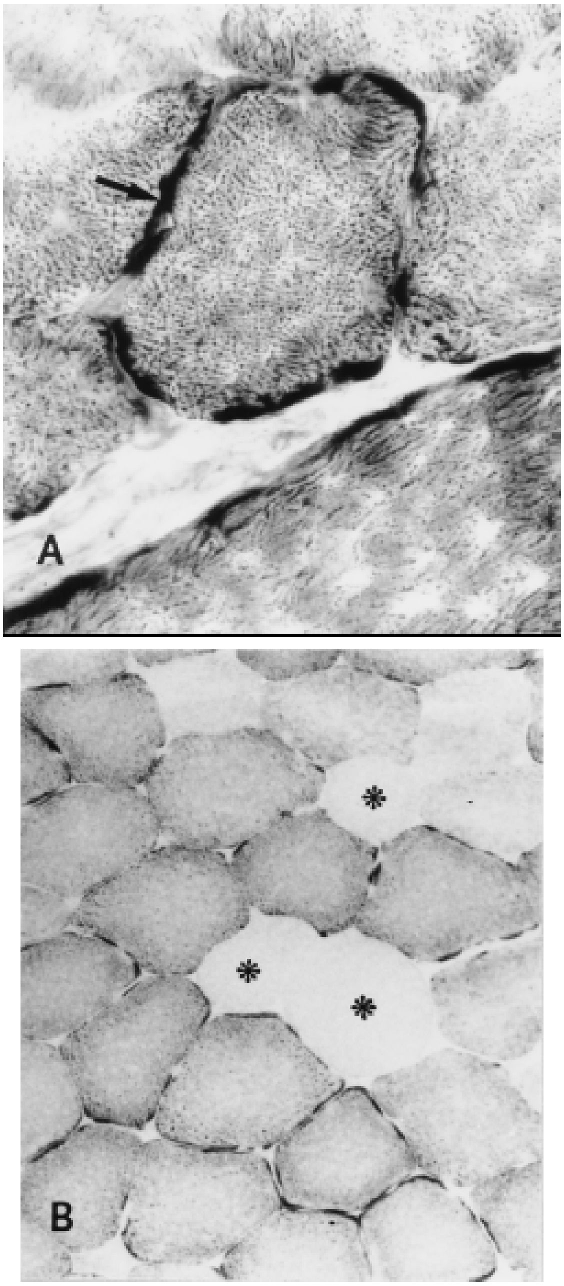

Fig. 2. Histochemical investigation of the muscle biopsy of the proband. **A:** Muscle fiber with subsarcolemmal accumulation (arrow) of mitochondria (SDH stain; ×400). **B:** Cytochrome oxidase staining showing occasional negative fibers (asterisks; ×160).

fectious mononucleosis. No further episodes have been noted since.

Patient IV-20

He presented with myoglobinuria in 1962 at 13 years of age after a viral pharyngitis. There was no renal insufficiency. Appendectomy was performed at age 15 without any complication.

Patient V-6

He was hospitalized in 1983 at age 15 because of muscle pain and high temperature. Urinalysis showed *E. coli* infection, and the presence of myoglobin and erythrocytes. Acute pyelonephritis was diagnosed. Creatinine was 103 μmol/l, calcium 2.3 mmol/l, CK 80,000 U/l rising later to 213,600 U/l. Renal function remained normal. One year later, he had an episode of infectious mononucleosis with a temperature of 38°C, but without myoglobinuria.

Patient V-10

At the age of 9 (in 1980), he had influenza with high fever and was hospitalized because of muscle pains and dark urine. Serum ASAT was 1100 U/l, creatinine 70 μmol/l and CK 97,900 U/l. There was myoglobinuria and ketonuria. CK levels returned to normal after oral rehydration. He has had no relapse since.

DISCUSSION

We have presented a family in which there was clear evidence for autosomal dominant transmission of myoglobinuria—males and females were affected, in 4 successive generations, with transmission by both sexes (including male to male). In addition, there are 3 instances of transmission by apparently unaffected individuals, suggesting reduced penetrance.

In the majority of cases, myoglobinuria occurred in the context of acute febrile viral or bacterial infection, and was never exercise-induced. The responsible pathogen was identified in 4 cases: *E. coli, S. pneumoniae,* Epstein-Barr virus, and influenza B virus. Four patients underwent general anesthesia without complication. In two instances, myoglobinuria led to acute renal failure that was reversible after dialysis. None of the affected individuals complained of muscle pain or fatigue, except during acute episodes. CK levels were normal between episodes.

Myoglobinuria leading to acute renal failure can be induced by viral or bacterial infections [Shenouda and Hatch, 1976; Henrich et al., 1980; Spataro and Marone, 1993]. Rhabdomyolysis could be due either to virus-induced myositis, as described after influenza A infection [Minow et al., 1974], or to the release of interleukin-1, which can stimulate protein degradation and activate the oxidation of branched-chain amino acids [Baracos et al., 1983; Nawabi et al., 1990]. However, as infections are very frequent compared to myoglobinuria, it is likely that there exist genetic conditions predisposing to rhabdomyolysis, which can be triggered by fever-induced metabolic changes.

Only one other family with autosomal dominant transmission of myoglobinuria has been reported to our knowledge [Kittur et al., 1987]. Myoglobinuria was precipitated not only by fever or viral infection, but also by prolonged exercise or alcohol abuse. CK levels were elevated between episodes. The pathogenesis could not be clarified, despite exhaustive histochemical and biochemical investigation.

In a study of 77 patients presenting with myoglobinuria, extensive biochemical investigations identified a metabolic disorder in only 46% of cases [Tonin et al., 1990].

Malignant hyperthermia is one of the rare established causes of autosomal dominant myoglobinuria.

Acute episodes in predisposed individuals are triggered by general anesthesia, and are characterized by an accelerated metabolism and muscle rigidity, leading to malignant hyperthermia and rhabdomyolysis. In some families, the underlying cause is a mutation of the ryanodine receptor gene, which leads to a defect in the closing of the calcium channel [Joffe et al., 1992]. Some cases have been associated with febrile illness [Poels et al., 1991]. In the family reported here, two affected individuals (IV-4 and IV-20) underwent general anesthesia without complication. This, combined with the presence of exercise-induced hyperlactatemia in the propositus, makes the diagnosis of malignant hyperthermia very unlikely.

Disorders of glycogen metabolism such as McArdle disease are most improbable in the current family, because of i) their autosomal recessive inheritance, ii) the absence of increased lactate on exercise in these disorders [Poels and Gabreëls, 1993], iii) the normal muscle glycogen content in the muscle biopsies of the proband and patient IV-2, and iv) the lack of association between myoglobinuria and exercise.

Myoglobinuria can also be a sign of mitochondrial fatty acid oxidation disorders and is typically triggered by prolonged exercise and fasting. Within this group of disorders, the adult form of CPT II deficiency [DiMauro and DiMauro, 1973] was initially suspected, but could be excluded because of its recessive mode of inheritance and the normal fibroblast CPT II enzyme activity in the propositus.

Long-chain acyl-CoA dehydrogenase deficiency, a defect of the fatty acid β-oxidation spiral, has been identified in 15 patients [Roe and Coates, 1995]. While the majority of cases present neonatally or in early infancy with fasting-induced coma and hypoketotic hypoglycemia, 3 adult cases have been described with bouts of muscle pain and myoglobinuria. In 1 case, the episodes were associated with viral infections and CK levels were elevated during the acute phases. This diagnosis is highly unlikely in the current family because of its recessive mode of inheritance, although it has not been formally excluded by metabolic screening.

Rhabdomyolysis and myoglobinuria have also been recognized as infrequent clinical signs in respiratory chain disorders. In the propositus, the presence of cytochrome C oxidase-negative (COX-negative) fibers in the muscle biopsy and of a subsarcolemmal accumulation of mitochondria is compatible with and points to such a diagnosis. The increase on exercise of the serum lactate and lactate/pyruvate ratio further supports this hypothesis. Ohno et al. [1991] described two brothers with recurrent myoglobinuria following exertion or alcohol intake, who similarly had increased serum lactate after exercise; muscle biopsies showed ragged red fibers and COX-negative fibers. Their muscle mtDNA contained multiple deletions, which are known to be secondary to dominantly-inherited nuclear mutations, at least in some cases [Suomalainen et al., 1995]. Such deletions were found neither in the muscle or blood of our index-case nor in leukocyte DNA from his living affected relatives. However, only a single muscle biopsy has been tested, and a false-negative result is always possible because of potential heteroplasmy.

In addition, Ogosahara et al. [1989] and Hirano et al. [1996] have described three individuals with encephalomyopathy associated with myoglobinuria and lactic acidosis, due to a deficiency of coenzyme Q10. We have not determined coenzyme Q10 levels in affected family members, but the absence of central nervous system involvement, the later onset, and the milder course make this hypothesis unlikely.

With the exception of complex II, the enzyme complexes which make up the respiratory chain are encoded by two separate genomes, the nuclear and the mitochondrial. Cytochrome C oxidase (complex IV) is composed of 13 subunits, of which three are encoded by the mtDNA [Morris, 1993]. Two cases of recurrent myoglobinuria due to cytochrome C oxidase deficiency have recently been reported [Saunier et al., 1995; Keightley et al., 1996]. In one case, a 15 base pair deletion in the mitochondrially-encoded gene for subunit III of cytochrome C oxidase was identified [Keightley et al., 1996].

Because of the Mendelian, rather than matrilineal, inheritance of the disorder in the current family, we postulate the presence of a mutation in a nuclear gene involved in mitochondrial function or biogenesis. As the family is too small for classic linkage analysis, it will be necessary to await the identification of suitable candidate genes to progress in the elucidation of the pathogenesis of the disorder.

In conclusion, this report illustrates the importance of mitochondrial investigations and exhaustive pedigree analysis when faced with a patient with unexplained myoglobinuria.

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